Novel nanostructured lipid carriers dedicated to nucleic acid delivery for RNAi purposes

Jonathan Bruniaux, Eric Sulpice, Frédérique Mittler, Mathilde Menneteau, Thierry Bastogne, Isabelle Texier, Xavier Gidrol, Fabrice Navarro

Abstract Nanotech

Novel nanostructured lipid carriers dedicated to nucleic acid delivery for RNAi purposes

J.Bruniaux[1, 2, 3, 4], E.Sulpice[2, 3, 4], F.Mittler[2, 3, 4], M.Menneteau[1], T.Bastogne[5, 6], I.Texier[1], X.Gidrol[2, 3, 4] and F.Navarro[1]

[1] CEA, LETI-MINATEC, Département des Technologies pour la Biologie et la Santé, 17 rue des Martyrs, F-38054 Grenoble, France
[2] CEA, DSV iRTSV, Biologie à Grande Echelle, Biomics, 17 rue des Martyrs, F-38054 Grenoble, France
[3] INSERM, U1038, F-38054 Grenoble, France
[4] Université Joseph Fourier-Grenoble I, U1038, F-38041, France
[5] Université de Lorraine, CRAN, UMR 7039, Campus Science, Vandoeuvre-les-Nancy, 54506, France
[6] CNRS, CRAN, UMR 7039, France

e-mail : jonathan.bruniaux@cea.fr
phone : (+33)4 38 78 24 18

RNA interference discovery has opened up new fields of study, including the understanding of dysregulation occurring in pathogenesis, especially in cancerogenesis. Through this mechanism, the controlled specific down-regulation improves the identification of gene function involved in a biological mechanism and their contribution in an altered phenotype[1]. In this way, high-throughput screening (HTS) of RNAi has emerged as a potent tool in order to study a large number of gene functions for the identification of new biomarkers and/or therapeutic targets. However, HTS requires for that purpose generic carriers with highly efficient transfection. Recently, progresses have been made regarding the design of non-viral vectors (*lipids, peptides, polymers*) as delivery agents[2]. The efficiency and specificity of gene-based expression of siRNA have been improved, while at the same time reducing toxicity. Among these carriers, lipid-based agents, already used as commercial reagents for *in vitro* transfection, are considered as a promising approach for siRNA delivery.

Here, we have developed novel nanostructured lipid carriers through a design of experiment approach which promote high transfection efficiency in several human cell lines. These lipid nanoparticles present an outstanding colloidal stability, whereas their cationic shell promotes ready-to-use features for fast and simple complexation with nucleic acids by electrostatic bonds establishment, without inducing cytotoxicity. As evidenced by gel retardation assay, complexation is stable over time, even in cell culture media, enabling thus stable transfection efficiency for all samples in a protocol of large-scale screening. Compared to the commercially available lipoplexes, these lipid nanoemulsions offer the possibility to combine several functions into a unique carrier, such as fluorescent particle tracking through the encapsulation of lipophilic dyes and/or the co-delivery of other drugs[3]. Thereby, the distribution monitoring into cells with nanocarriers entrapping lipophilic cyanine derivatives demonstrates an improved internalization of nucleic acids. Furthermore, preliminary experiments performed on primary cells in culture demonstrated also an improved transfection compared to commercially available lipoplexes, opening thus the way for RNAi screening in that cells recognised as hard to transfect

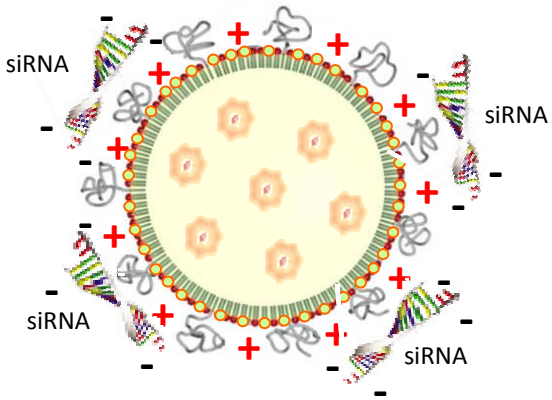
Besides, ligand moieties can be grafted onto the particle surface in order to selectively target a specific subset of cells. Such cationic lipid nanodroplets open new avenues in the development of high throughput RNAi screening as well as their translation to clinics.

Topic: Nanotech Tracks / Biotech & Pharma / Materials for Drug & Gene Delivery / Novel delivery system

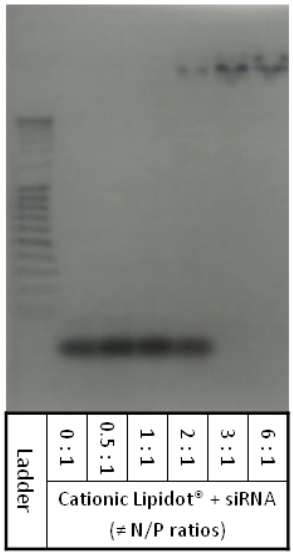
---

[1] Colombo, R. & Moll, J. Molecular diagnosis & therapy, *12*(2) 2008
[2] Kozielski *et al*, Wiley interdisciplinary reviews 2013
[3] Navarro *et al*, Journal of Biomedical Nanotechnology 8 2012

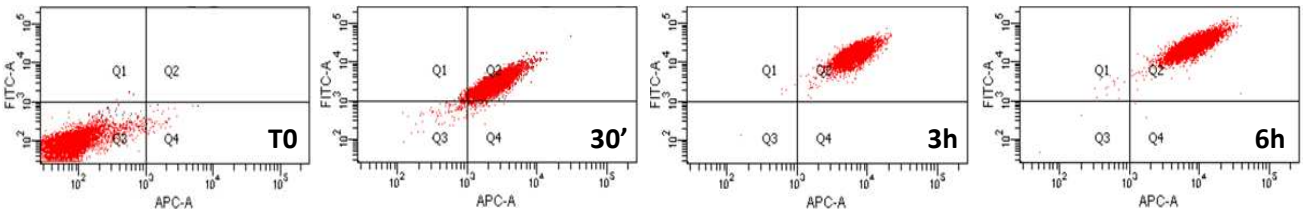
A/



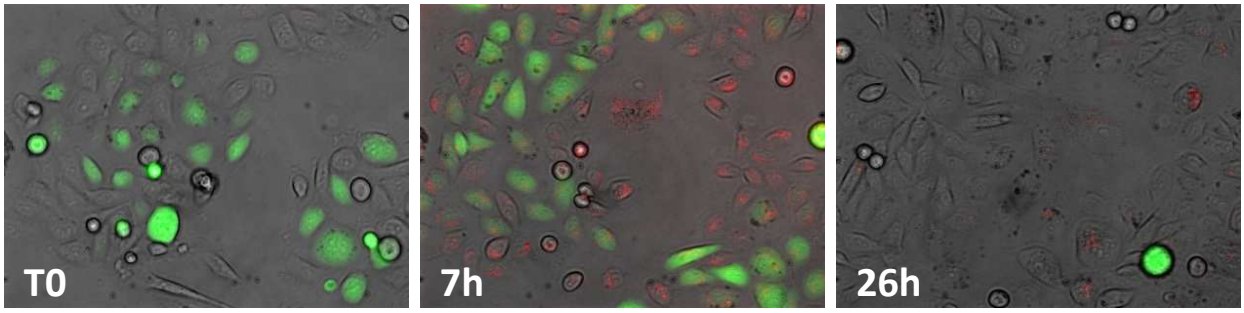
B/



C/



D/



A/ Structure of lipid nanoemulsions, namely Lipidots®, after incorporation of cationic compounds in order to complex negatively charged siRNA. B/ Gel retardation assay showing efficient complexation of siRNA on lipid nanoparticles. C/ Dot plots obtained by FACS displaying the fast internalisation of cationic lipid nanoparticles doped with DiD (APC-A) complexed with siRNA labelled with Alexa-488 (FITC-A) overtime. D) Epifluorescence imaging of prostatic cells overexpressing GFP protein (T0) after internalization of cationic Lipidot/siRNA complex (7h) and after specific down-regulation of GFP protein by siRNA (26h).